TOTAL RNA ISOLATION FROM TISSUE PROTOCOL

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TOTAL RNA ISOLATION FROM TISSUE PROTOCOL

1. Weigh tissue. Homogenize tissue in 50mg/ml RNA-Bee reagent, using Brinkman Tissuemizer for 30 to 60 seconds. If the lysate is not processed immediately, store at -70°C.
2. Centrifuge lysate for 5 minutes at 2000g.
3. Transfer supernatant to fresh tubes.
4. Add 0.2ml chloroform per 1ml lysate.
5. Vortex each tube for 15 seconds.
6. Store at room temperature for 15 minutes and then centrifuge for 20 minutes at 13,000g.
7. Transfer the aqueous phase in to fresh tube. Add 10ul of glycogen to the aqueous phase, add 0.25ml of 0.8 M NaCitrate/ 1.2 M NaCl high salt solution to each 1ml lysate, briefly vortex to mix well, then add 0.25ml of isopropanol to each 1ml lysate. Vortex 15 seconds to mix well.
8. Store at room temperature for 15 minutes.
9. Centrifuge for 20 min. at 13,000g @ 4°C. Remove supernatant. Air dry briefly.
10. Add 1 ml of 75% ethanol to each 2ml lysate, briefly vortex to mix, spin for 20min at 13,000g at 4°C. Air dry
11. Rehydrate RNA pellet in DEPC water (amount depends on the RNA pellet size). Mix well.
12. Read OD in 10mM Tris HCl pH7.5 after 1:100 dilution
13. Calculate the RNA concentration
14. Take out about 20 ul for cDNA and chip analysis and store @ -70°C
15. Re-precipitate RNA by adding 1/10 volume of 3 M Sodium Acetate and 2.5-volume alcohol and store at –20°C.

EXAMPLE OF DATA ACQUISITION AND ANALYSIS